# Probabilistic Risk-Based Model: *Assessment of Phytosanitary Risk Reduction Associated with Seed Quality Management Practices*



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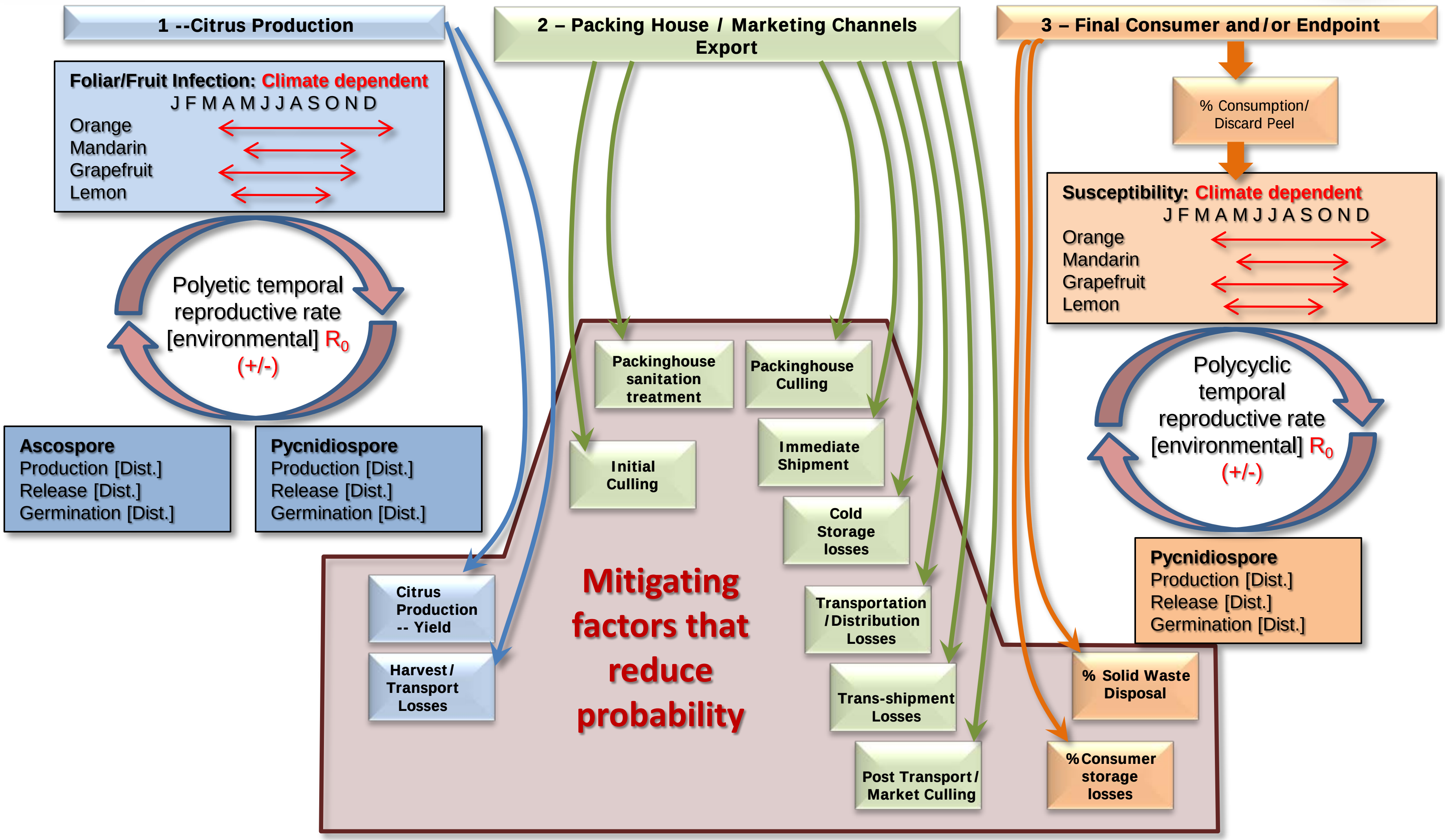
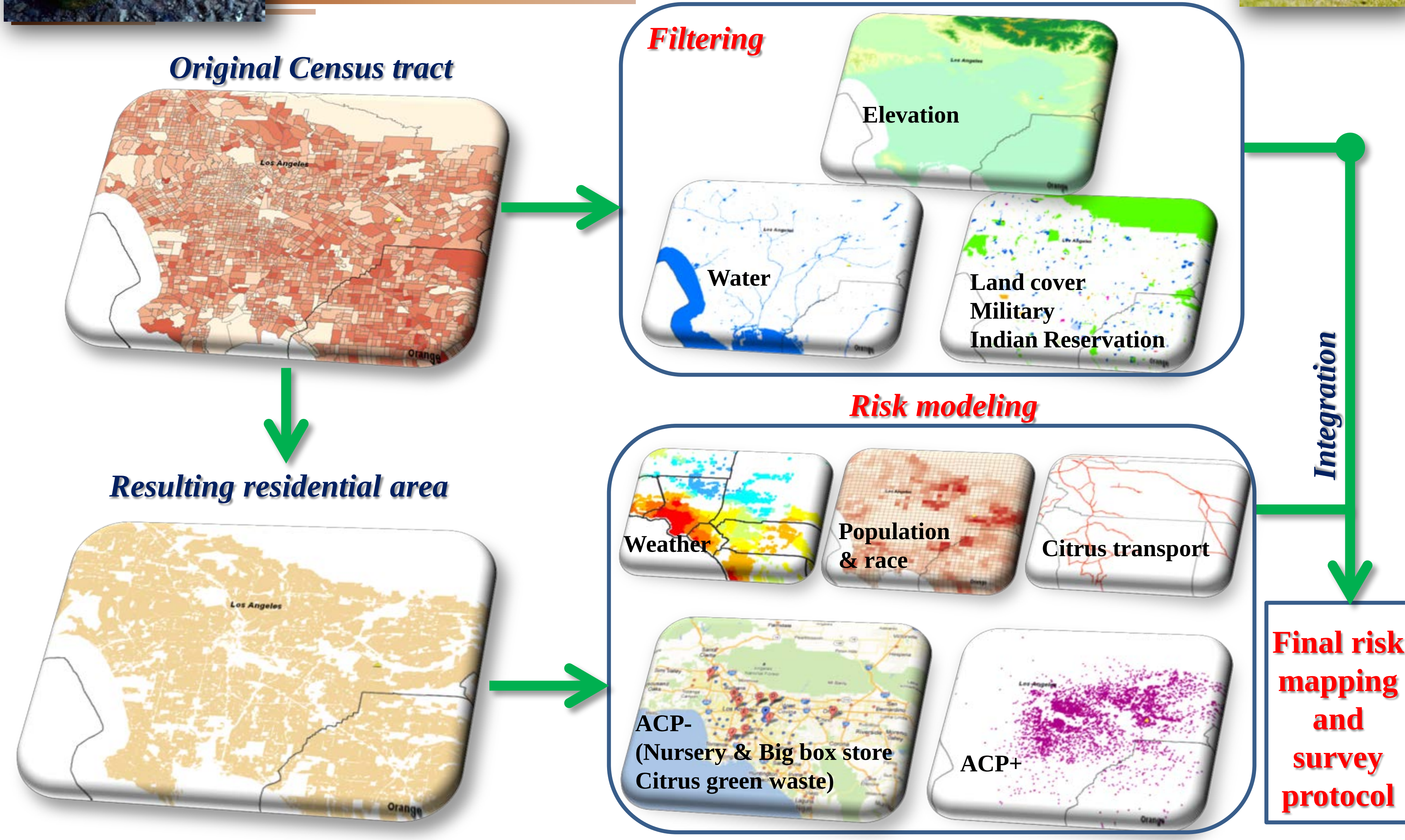
# Examples of Applied Probabilistic risk assessment models

1. **Citrus Black Spot:** Probabilistic Risk-based Model for International Citrus Fruit Trade Security
2. **Citrus Huanglongbing (HLB):** Risk-based Residential and Commercial HLB/Asian Citrus Psyllid Survey for California, Texas, and Arizona
3. **US Census/International Travel Survey:** Risk-based Targeted Survey via GIS Mapping to predict points of introduction of Exotic Plant, Animal and Human pathogens
4. **Plum Pox Virus (PPV):** Risk-based Survey Model for early detection and regulatory intervention

# Citrus Black Spot Probabilistic Risk-based Model for International Trade Security: *Examine risk of fruit as a pathway vector for introduction to new areas, ex. FL to EU*



# Citrus Huanglongbing (HLB): Risk-based Residential and Commercial HLB/Asian Citrus Psyllid Survey for California, Texas, and Arizona




www.plantmanagementnetwork.org/edcenter/seminars/Outreach/Citrus/HLB

# US Census/International Travel Survey – Predictive Risk Mapping Model for Exotic Plant, Animal and Human Disease/Pest Introductions *(Birth Country:Travel Bias = 1:3)*

| Count | Total CITORI Value for | CITORI as porportion of |
|---|---|---|
| Sum of BR | | |
| Sum of ME | | |
| Sum of JAM | | |
| Sum of DO | | |
| Sum of CU | | |
| Sum of HA | | |
| Sum of JAP | | |
| Sum of CH | | |
| Sum of IND | | |
| Sum of PH | | |
| Sum of TAI | | |
| Sum of HO | | |
| Sum of VIE | | |
| Sum of PA | | |
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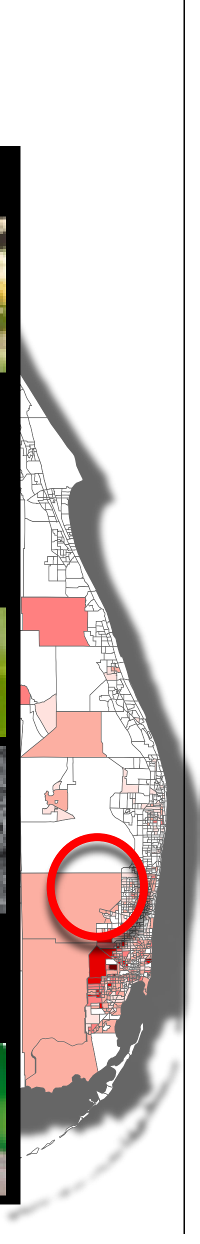
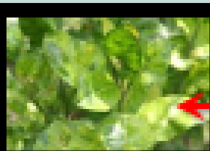
| Sum of BA | | |
| Sum of LAC | | |
| Sum of THA | | |
| Sum of SO | | |



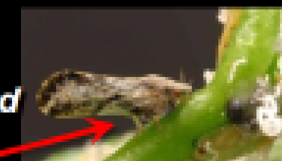
## Census-Travel Survey - Exotic Pathogens/Vectors Considered

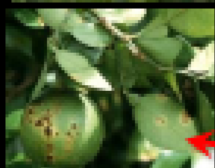


**Huanglongbing (HLB):**

Pathogens: Bacteria *Liberibacter asiaticus, L. africanus, and L. americanus*

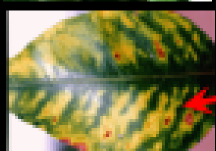
Vectors: Psyllids *Diaphorina citri* and *Triosa erytrae.*





**Pathogen: *Xanthomonas citri* subsp. *citri***

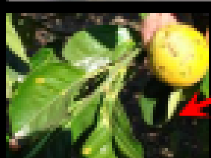
Spread: windblown rain and man.



**Citrus Variegated chlorosis (CVC):**

Pathogen: Bacterium *Xylella fastidiosa*

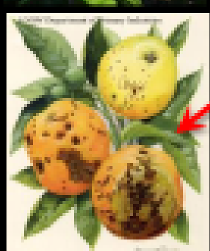
Vector: cicadellidae species (sharpshooter)



**Citrus Leprosis:**

Pathogen: Citrus Leprosis virus (CiLV)

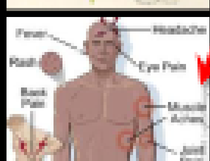
Vector: mite *Brevipalpus phoenicis*



**Citrus Black Spot (CBS):**

Pathogen: ascomycete fungus, *Guignardia citricarpa*

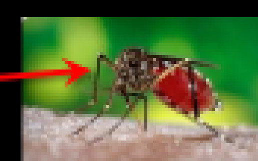
Spread: rain splash of inoculum.




**Several Human/animal diseases: Ex. Dengue fever:**

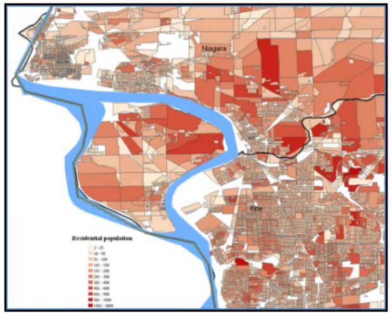
Pathogen: *Flavivirus* (DENV)

Vector: mosquito, *Aedes aegypti*



# Plum Pox Virus (PPV): Risk-based Survey Model for early detection and regulatory intervention

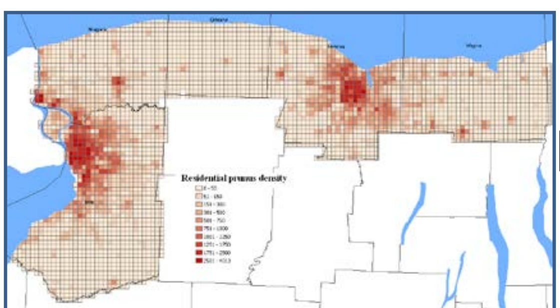
## Census blocks



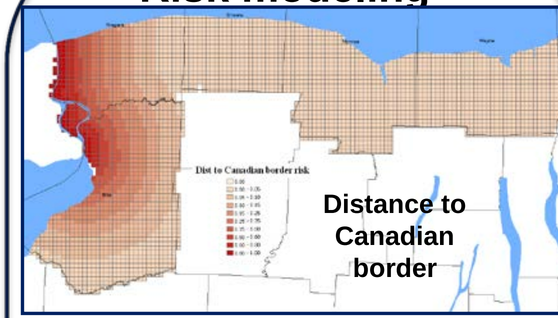
## Filtering

- Military installation
- National parks
- Commercial areas
- Etc.

## Residential *Prunus* estimation



## Risk modeling

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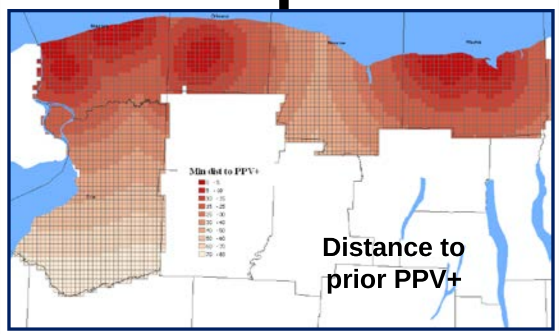


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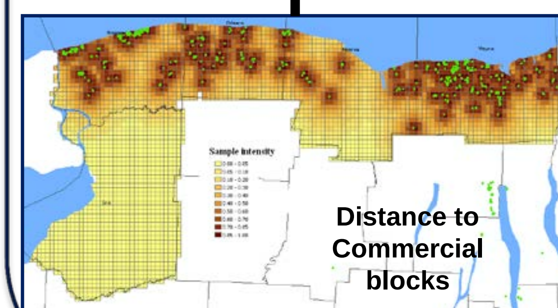
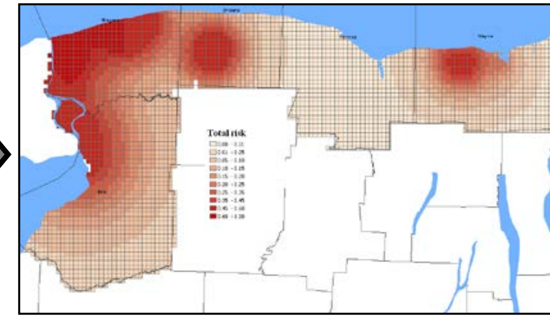
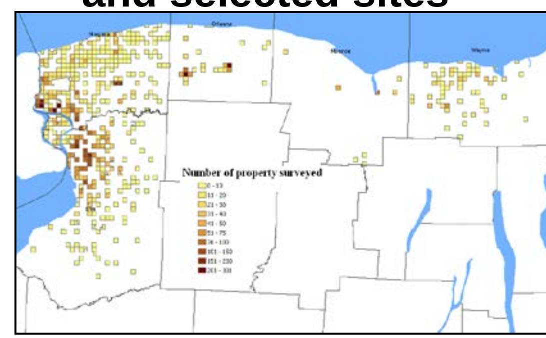



## Final risk mapping



## Final survey protocol and selected sites



## Deploy to Regulatory Agencies

ArcMap layer files, Excel file with risk rankings


# Probabilistic Risk-Based Model to Assess Seed *Phytosanitary Risk Reduction*

## Motivation

- **Consumers expect healthy, disease-free seeds.**
- **Identify and optimize phytosanitary issues: Costly and damaging to the entire seed industry when are not timely identified.**
- ***Aid in the development of international phytosanitary standards to support a more predictable trade environment.***

## Expected Outcomes

- **Method to quantitatively assess how steps in production practices reduce phytosanitary risks.**
- **General framework that can be applied to any plant system (pathosystem).**
- **Framework on which to develop/justify international phytosanitary standards**


# In general follows the Guide to seed quality management practices (*qualitative*)

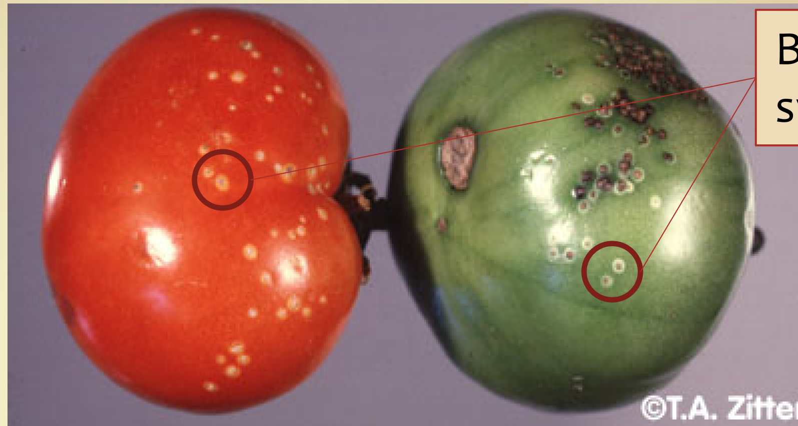
- Created by ASTA in July 2010.
- Step-wise guidance for developing quality management practices.
- Follows Hazard Analysis and Critical Control Points (HACCP) principles.
- Eight modules from incorporation of a trait into a breeding program through commercial seed production and sale.

- How does following quality management practices affect phytosanitary risk concerns?

# Proposed Risk model Pathosystems - Tomato

## 1. *Clavibacter michiganensis* subsp. *michiganensis* (Cmm)

Very complex system:
- Multiple tomato production methods.
- Cmm can survive for long periods under broad conditions.
- Tomato infected with Cmm may remain asymptomatic for some time.




Bacterial canker symptoms on fruit

- Survival possible in soil, plant debris, weed hosts, volunteer plants, and seed.
- Dispersal through wind and water.

## 2. *Potato spindle tuber viroid* (PSTVd) – *On Tomato!*

- Mechanical transmission
- Frequency of seed transmission appears uncertain at this time.

# General seed production pathway

**3 possible greenhouse (SOPs) Pathway branches:**

1. **GSPP (good seed and plant practices)**
2. **Non-GSPP, but well managed. Can vary risk due to materials, people, and water**
3. **Poorly managed.**

**Pathway branches resulting in 3 different risk outcomes!**

Breeding material
- Clean parent/donor material

Breeding program
- Greenhouse
- Laboratory
- Field

**Branching due to breeding method used:**

**conducted in the greenhouse**
**laboratory**
**field settings**

**Each has different phytosanitary concerns.**

Field testing
- Variety and trait testing
- Breeder seed and seed stock development

Continual product integrity
- All aspects of production management

Commercial sale


# Benefits of risk assessment model

- **Formal procedure to assess the significance of risks and an input into decision making.**
- **Quantification of each step in the seed pathway.**
- **Identify phytosanitary issues quickly (high risk outcomes).**
- **Numerically test how changing production practices will affect phytosanitary risks.**
- **Compare critical control points (i.e., which critical control points are more critical than others? Which contribute most risk!)**
- **Effort spent on preventative measures will lesson reliance on crisis management.**

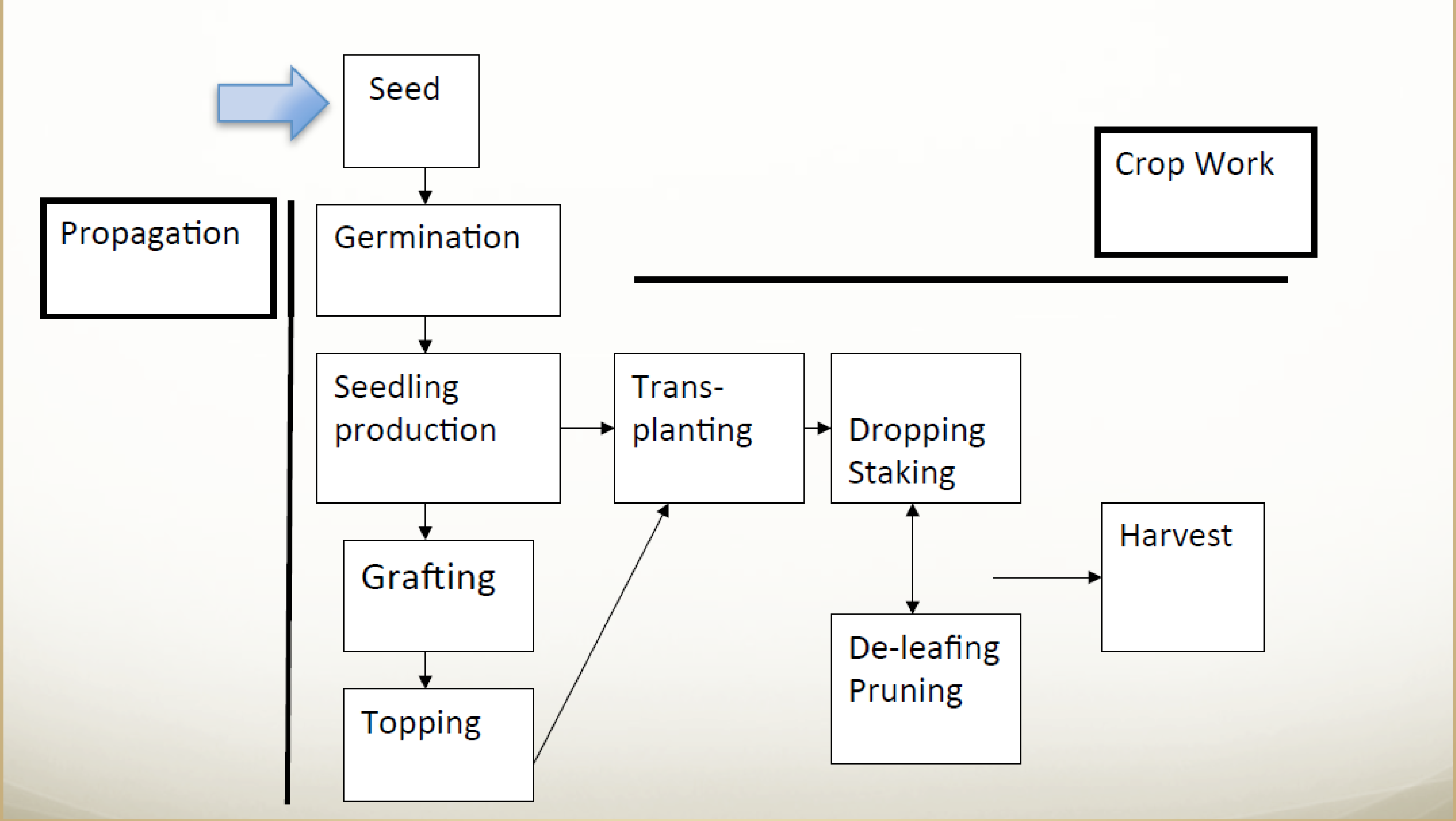

# Goes beyond HACCP

- Identify steps in the pathway that contribute the highest amount of risk (sensitivity analysis).
- Anticipation and contingency planning; *'what-if'* scenarios.
  - Can test any scenario and estimate risk reduction or increase.
- Discover steps in the pathway that can be adjusted to reduce risk, and the amount of reduction that would be expected due to the change implemented.


# Data Sources

- **Data mining of Published Literature**
  - **Much is available**
- **Acquire data directly from seed production companies**
  - **Some production methods may be specific to individual company**
  - **Need data resulting from specific method application**
- **Where no data is available:**
  - **Define precise missing data**
  - **Design and conduct experiments to fill data gap**
  - **Analyze data and use to populate model**

# Example: Greenhouse tomato production flow (from website) – one possible pathway




- Individual companies and situations will vary of course.
- We will try to capture these variations

# Example: Known data for Bacterial canker (cmm) control efficacy - extracted from the scientific literature

- Seeds germinate under environmental conditions (warm temperature, high moisture) highly favorable to Cmm

- >$10^6$ cfu/g seedling leaves required for epidemic in field
  - Shirakwawa et al. JARQ 25:27

Climatic conditions

Grafting

- Rootstock and scion = double chance of Cmm introduction

- Contaminated hands, tools, clothing spread pathogens during grafting and topping

- Grafting tools readily transmit Cmm but an inoculum threshold approx. $10^2$-$10^4$ cfu/ml may be required

**Phytosanitary Risk**

Initial disease level

Seed treatment

- Numbers of Cmm necessary to affect seed health and transmission are small (A. Alvarez)
  - As few as 25 cfu/seed inhibit germination
  - As few as 600 cfu/seed kill seedlings
  - Infestations of seeds within a seed lot are variable

- 1-5 infested seeds in 10,000 sufficient to start epidemic
  - Chang et al. Phytopathology 81: 1276

## Seed Treatment Efficacy

| Treatment | Cmm-infested seed ( % ) | Seed Vigor Index |
|---|---|---|
| Control | 11.8 b | 801 ab |
| Kasugamycin | 10.1 bc | 603 c |
| $H_2O_2$ | 15.3 a | 803 ab |
| Streptomycin | 6.3 d | 766 b |
| Thymol | 2.3 ef | 747 c |
| Dry heat | 7.6 cd | 536 d |
| Hot water | - | 785 b |
| KleenGrow | 0 f | 797 ab |
| Virkon-S | 0 f | 789 b |
| NaClO 50°C | 0 f | 778 b |
| HCl | 0 f | 833 a |


# *Methodology:*

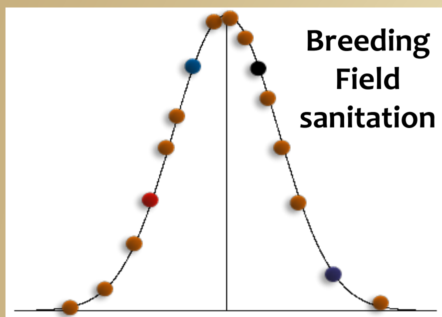
# Risk Modeling

## to determine risks associated with each step in the pathway

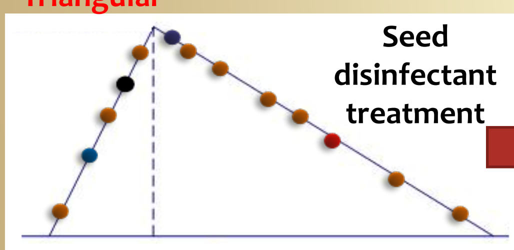
# Monte Carlo simulation

## Example Input variable distributions

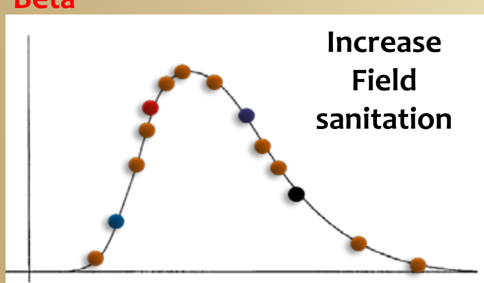
Normal

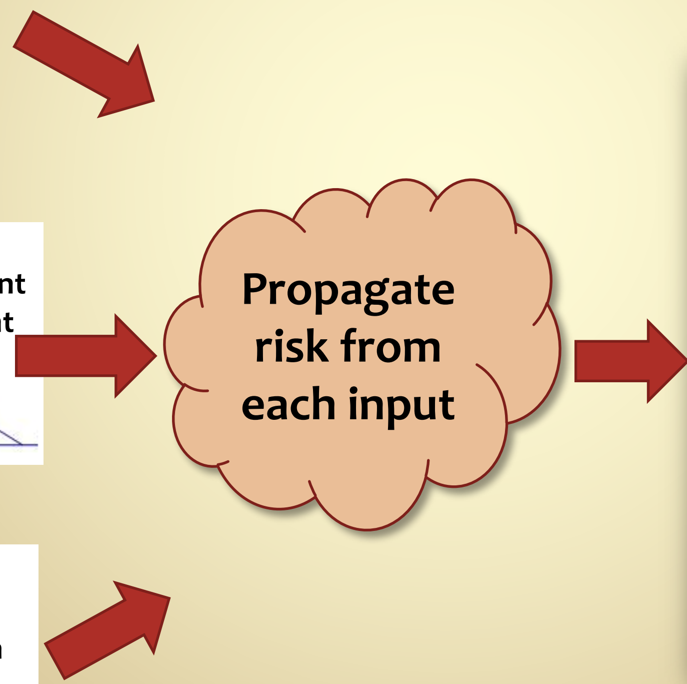



Triangular



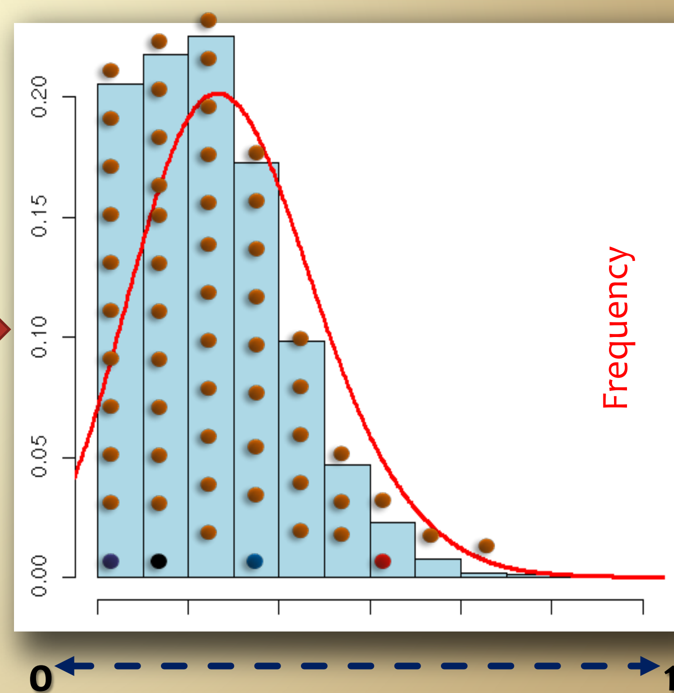

Beta




## Output distribution

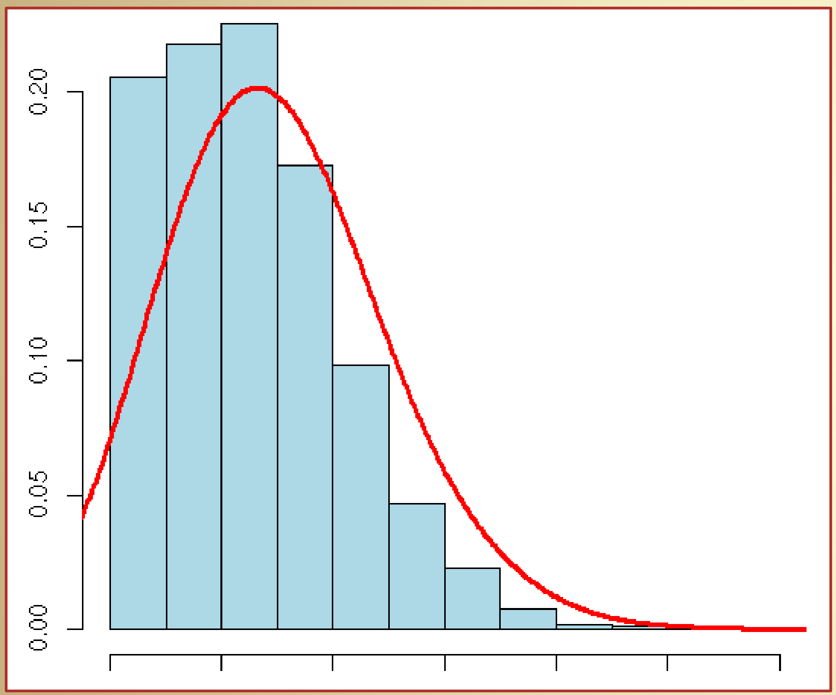
Result of 10,000 to 100,000 simulations




Accumulated Risk

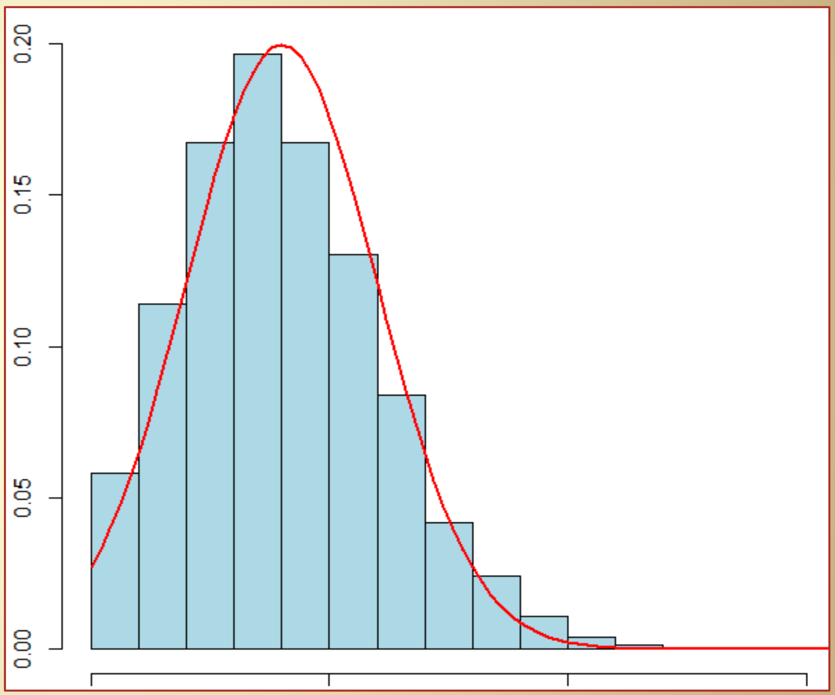
# 'what if' scenarios

RISK OF **LOW** DISEASE PRESSURE ENVIRONMENT



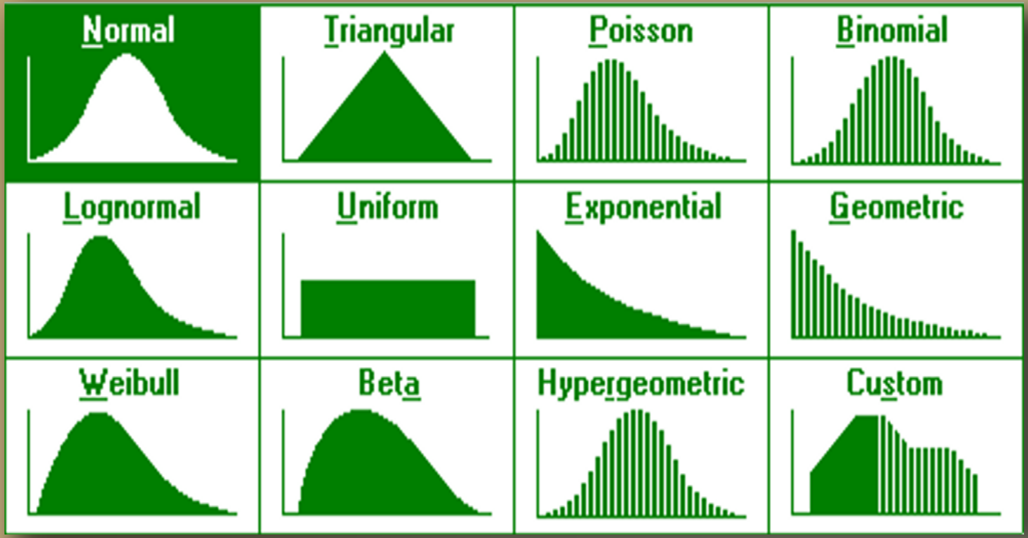

Risk

RISK OF **HIGH** DISEASE PRESSURE ENVIRONMENT




Risk

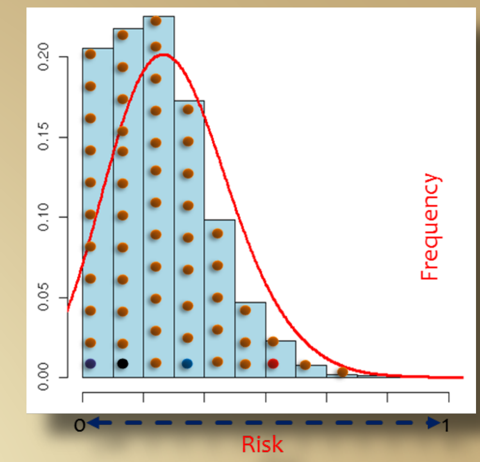
Output shifted to the right – higher risk




# Monte Carlo simulation

- A statistical technique used to model probabilistic (or "stochastic") systems and establish the odds for a variety of outcomes.
- Allows for complex interactions among several variables.
- Uses random input distributions (with realistic limits) with different input distributions.
- With our computer systems, minimal time needed to run large numbers of iterations which improves accuracy of output distributions.
- Highlights the relative impact of each variable.

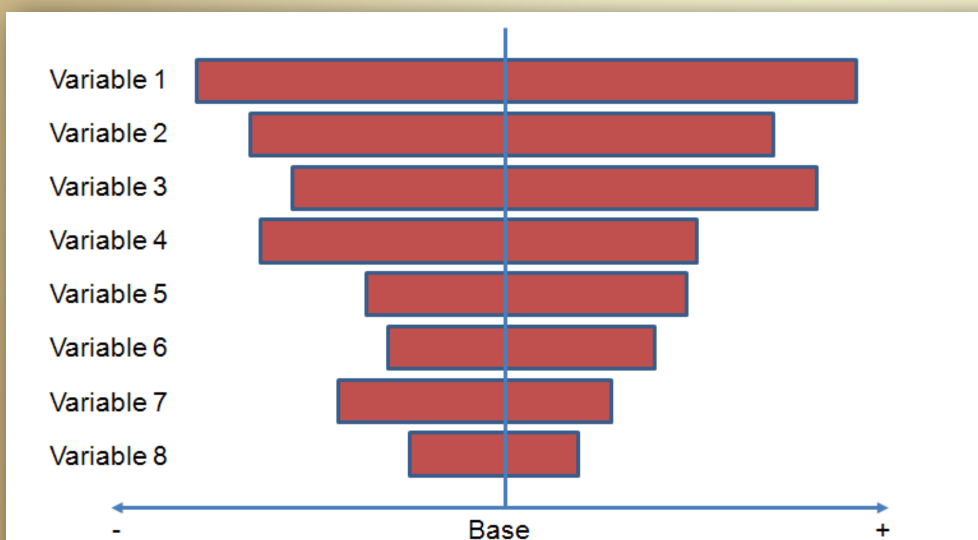

# Output distributions




- **Frequency**: Number/proportion of non-clean "infected" seeds per unit (lot, bulk, etc.).
- **Severity**: Concentration of infection per unit.
  - For some phyto sanitary concerns, some maximum threshold may be allowable (i.e., severity threshold below which the product is suitable).
- **Detectability**: Correction factor for probability of infection prior to detection.
  - Some pathogens, such as Cmm, may yield asymptomatic (nondetectable) plants for some time.
- **Correctability**: Correction factor for feasibility of implementing corrective action.
  - Some hazards (employees not always handwashing) can be more easily (cheaper) mitigated than others (moving all production to GSPP-compliant greenhouses).


# Sensitivity analysis: *What steps in the pathway contribute the greatest risk (to target)?*

- Method to rank input variables by their influence on the final risk level.
- Comparisons among input variables are typically illustrated on a tornado plot.




Most influential

Risk

Least influential


# Model flexibility

- The Monte Carlo methods with sensitivity analysis can account for the entire seed pathway (breeding to sales) or for just variables that affect one portion (factors related to planting location).
- We suggest evaluating multiple subsets of variables when making risk management decision(s).
    - A variable may be the most influential in regards to a small portion of the whole pathway, but much less influential (and not worth the cost of mitigating) when the entire pathway is analyzed.
- Although each model is specific to a pathosystem (seed species and pathogen/pest), *the overarching framework is malleable* and can be adapted to other pathosystems as needed, i.e. to many seed production systems.


# Example: seed production operations - planting preparation

- **Some possible phytosanitary concerns in association with planting for tomato seed stock:**
  - **The nearest distance to a known Cmm infected plant.**
  - **Level of weed control in field, borders, and nearby fields.**
  - **Probability of infested soil.**
  - **Amount of plant debris in area.**
  - **Concentration of Cmm in irrigation water/system.**
  - **Level of contamination/disinfectant of any materials used for planting or pruning.**
  - **Number of times any contaminated material comes in contact with plant material.**
  - **Risks from production workers (hands, clothing).**
- **With supporting data (distributions), each of these scenarios (and many more) can be quantified and included in the risk assessment.**

# Risk-based assessment modules

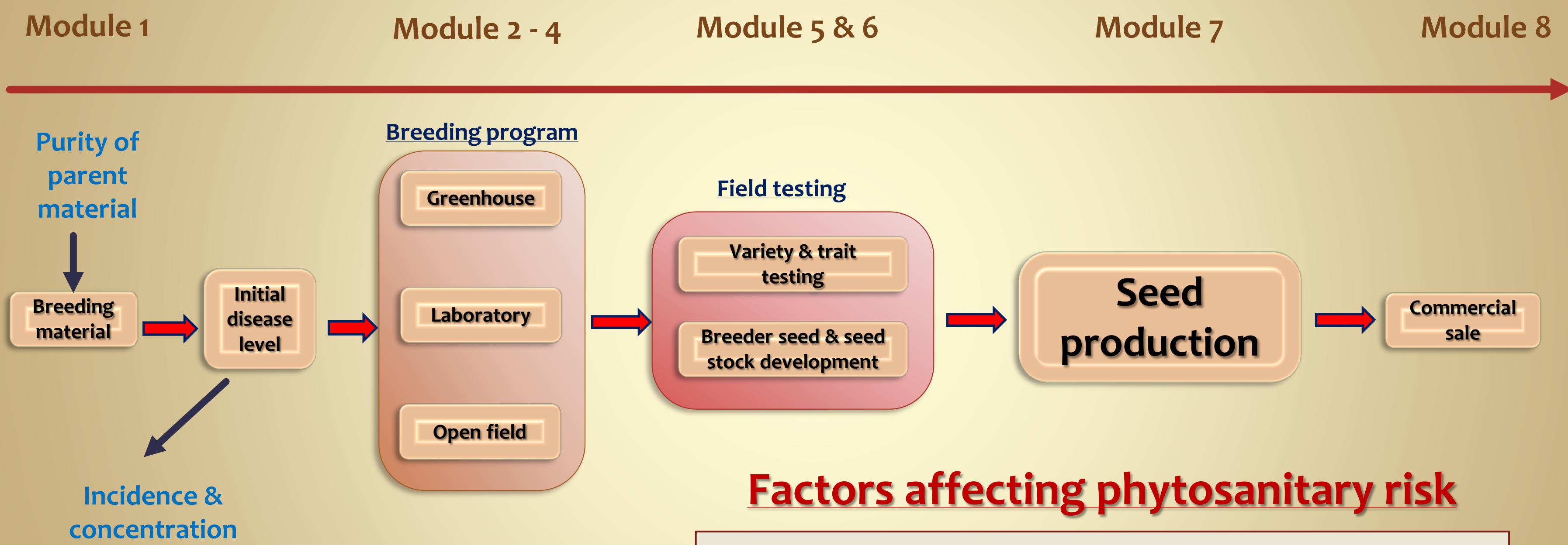
- ***Eight modules*** are considered from the point of incorporation of seed into breeding program to commercial seed production & sale.

**Module 1 – Incorporation of seed into breeding material**
**Module 2 – Greenhouse or other contained facility**
**Module 3 – Laboratory or storage facility**
**Module 4 – Field**

**Module 5 – Variety & trait testing**
**Module 6 – Breeder seed & seed stock development**
**Module 7 – Plant preparation and operations**
**Module 8 – Commercial seed sales**

- Aside from specific aspects of production, we are also interested in quality assurance/control tomato seed production guidelines individual companies utilize.
- Depending on specific protocols and production guidelines, individual modules may collapse to a single risk factor.
- A model will be designed in a way to accommodate various general seed business models & practices, and determine their final seed quality control performance by propagating risk from each module.

# We have begun to translate these modules into an initial model framework: Flow chart for Phytosanitary Risk modelling




## Factors affecting phytosanitary risk

- Environmental conditions for site, soil & water
- Cleanness of the transport vehicle & equipment
- GSPP/Non-GSPP managed
- Distance to known infection source
- Disease favorite climate variables controlled or not
- Cultural practice and disease monitor & control
- Harvest & post harvest infestation
- Seed extraction & cleaning
- Seed storage, warehousing & distribution

# Example Module 2: Tomato, Bacterial canker (cmm)

Isolation of seed/seedling production from the environment

Proportion

Initial disease level

GSPP greenhouse

Non-GSPP greenhouse

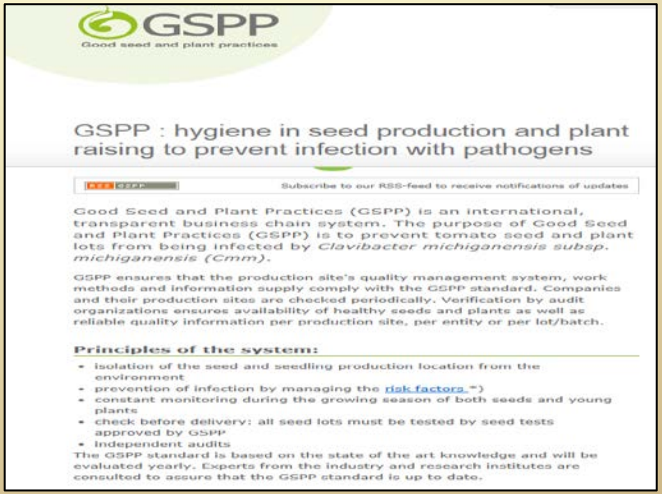
Well & poorly managed

Open field

## Risk propagation

- Distance to infection
- Splashing/irrigation water
- Climate conditions (RH & Temperature)
- Transmission by grafting & crop handling
- Constant monitoring during growing season
- Seed treatment
- Contamination from people & equipment
- Storage after harvest
- Etc.

Risk



GSPP
Good seed and plant practices

GSPP : hygiene in seed production and plant raising to prevent infection with pathogens

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Good Seed and Plant Practices (GSPP) is an international, transparent business chain system. The purpose of Good Seed and Plant Practices (GSPP) is to prevent tomato seed and plant lots from being infected by *Clavibacter michiganensis subsp. michiganensis (Cmm)*.

GSPP ensures that the production site's quality management system, work methods and information supply comply with the GSPP standard. Companies and their production sites are checked periodically. Verification by audit organizations ensures availability of healthy seeds and plants as well as reliable quality information per production site, per entity or per lot/batch.

**Principles of the system:**

- isolation of the seed and seedling production location from the environment
- prevention of infection by managing the risk factors *)
- constant monitoring during the growing season of both seeds and young plants
- check before delivery: all seed lots must be tested by seed tests approved by GSPP
- independent audits

The GSPP standard is based on the state of the art knowledge and will be evaluated yearly. Experts from the industry and research institutes are consulted to assure that the GSPP standard is up to date.


# Building the model: Step 1

- **Identify variables to include in the model.**
- **Need to rely on expert opinion, literature, and published/ not published data, proprietary.**
- ***Potential need for 'gap-filling' research!***

**Example: 5 variables for Cmm**

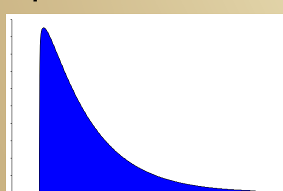
1. Distance to Cmm infected plants
2. Number of occurrences where pruning tools have Cmm
3. Cmm concentration in irrigation water
4. Plant debris (units) in nearby fields
5. Number of employees that forget to wash their hands


# Step 2

- **From available data or expert opinion, choose (statistically fit) the most appropriate input distribution to represent the risk associated with each variable.**

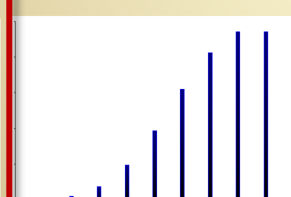
Distance to Cmm infected plants

Risk



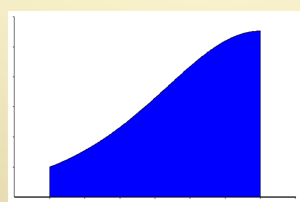
Gamma

Number of occurrences where pruning tools have Cmm



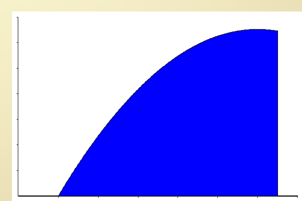
Poisson

Cmm concentration in irrigation water



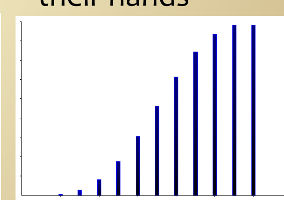
Logistic

Plant debris (units) in nearby fields

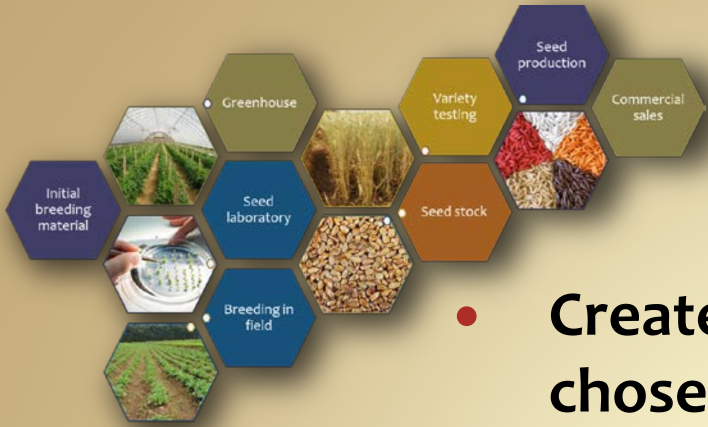


Beta

Number of employees that forget to wash their hands



Negative Binomial




# Step 3

- **Create a function for each input distribution to chose values in the Monte Carlo simulation.**

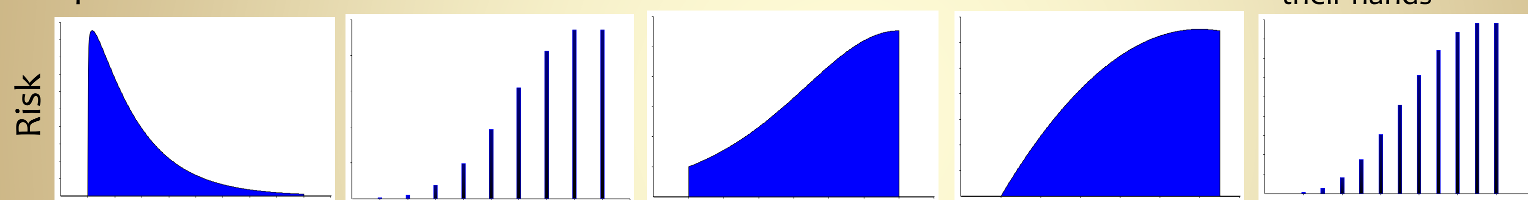
Distance to Cmm infected plants

Number of occurrences where pruning tools have Cmm

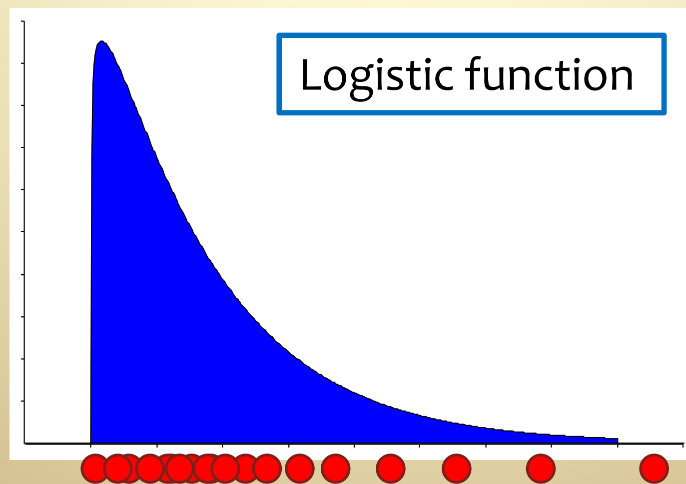
Cmm concentration in irrigation water

Plant debris (units) in nearby fields

Number of employees that forget to wash their hands



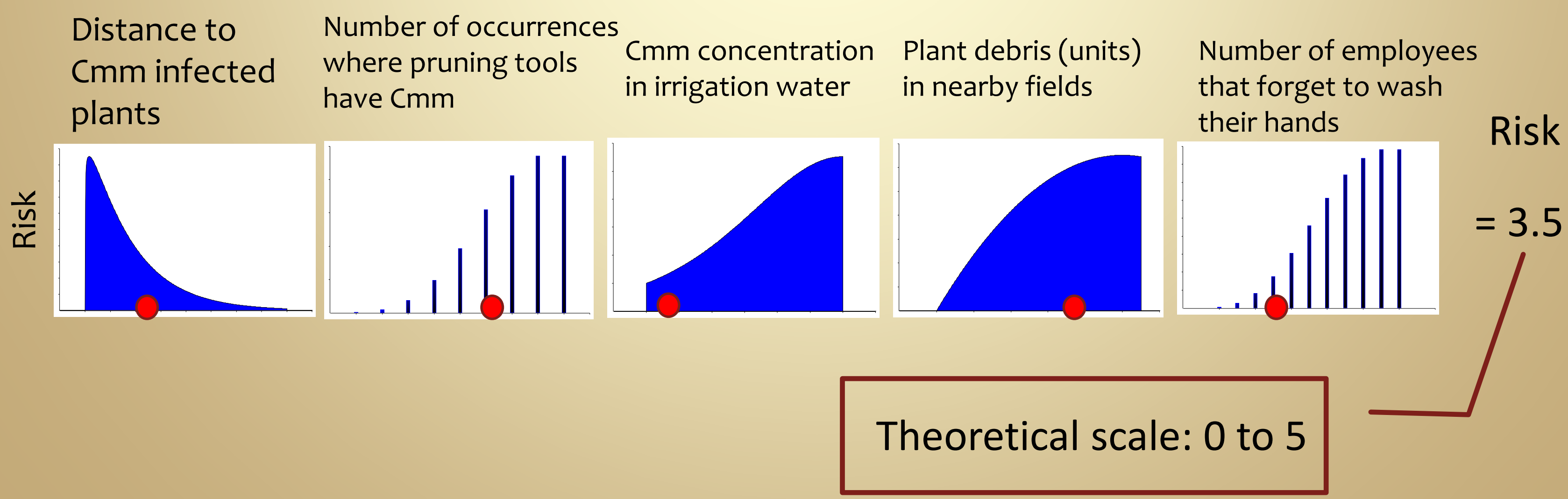

**Example:**




Distance to Cmm infected plants

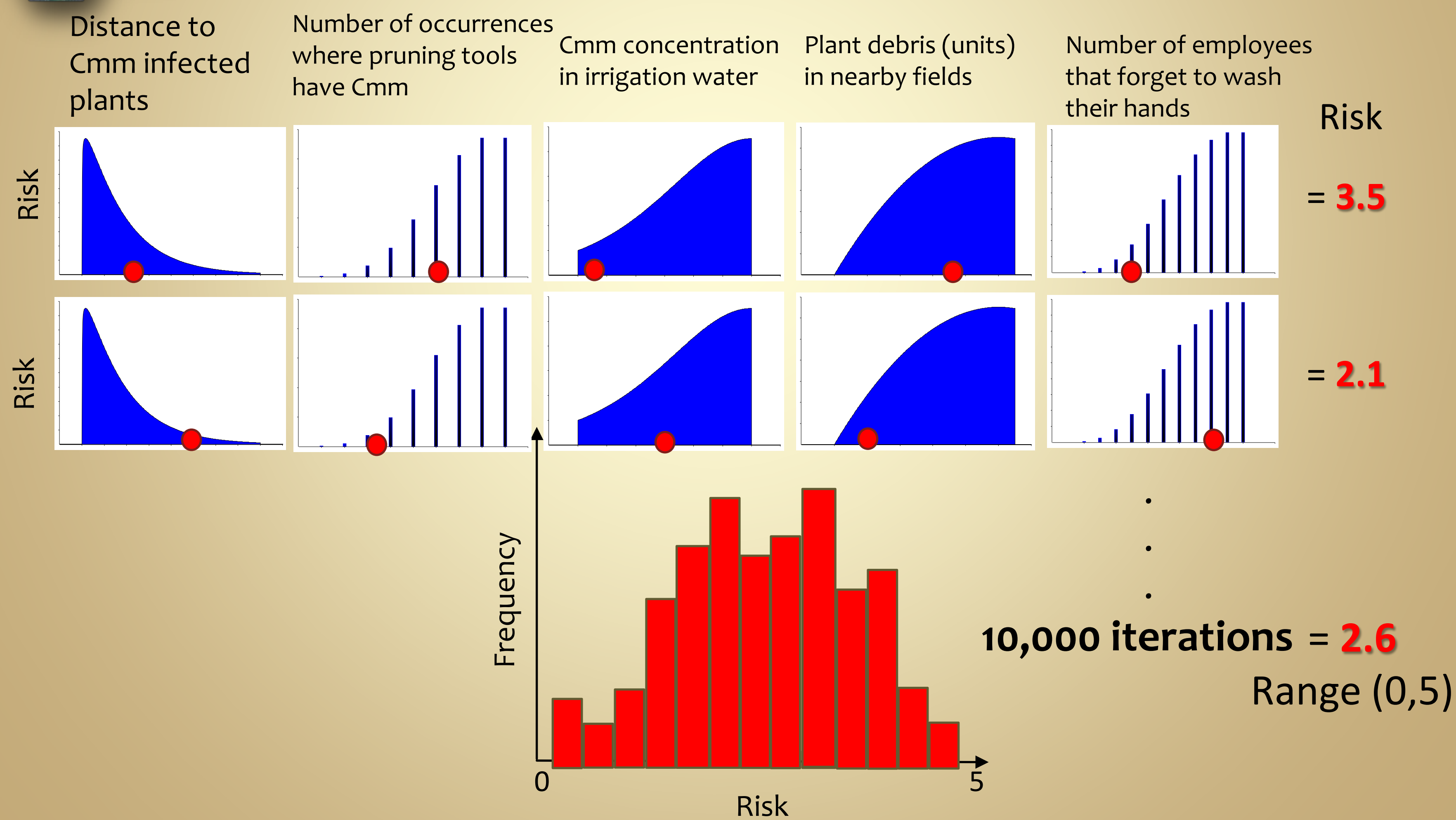

# Step 4

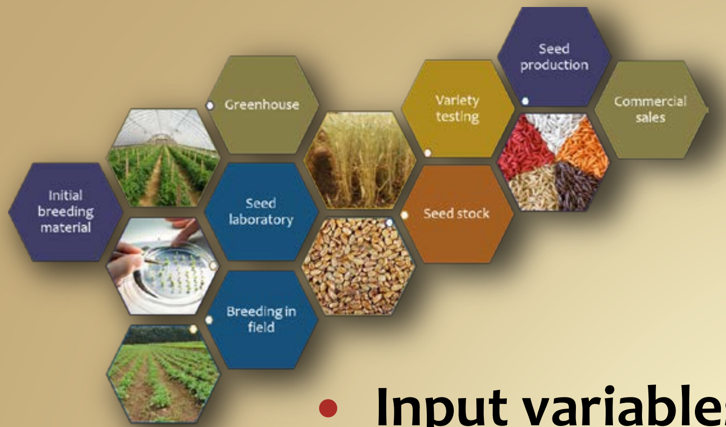
- In each iteration, the Monte Carlo simulation will choose a value from the function related to each input distribution.
- Complex systems and interactions among input variables area properly accounted for.
- Standardize resulting risk values.




# Step 5

- **Conduct multiple iterations (*ex: 10,000*) and examine results.**

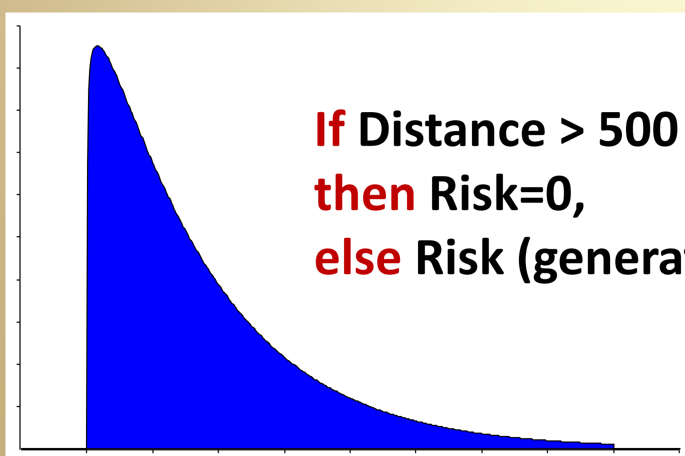





# Model complexities

- **Input variables can be independent or correlated with each other.**
  - **Risk from independent variables results is a simple summation (example).**
  - **Risk from correlated events can be weighted and multiplied.**
- **Critical control points that when implemented can reduce all possible risk to zero are implemented. Build in *If… Then… Else* step to halt model.**

**Example:**



**If Distance > 500 m,**
**then Risk=0,**
**else Risk (generated from the gamma distribution)**

Distance to Cmm infected plants


# ‘what if’ scenarios

- **What is the reduction in risk of Cmm contamination if a company (Company A) guarantees they only produce tomato seed in an environment far from any current sources of Cmm (i.e., guarantee a low disease pressure environment).**
- **Some variable are overarching, If risk =0, then entire pathway stops and overall pathway risk is 0.**

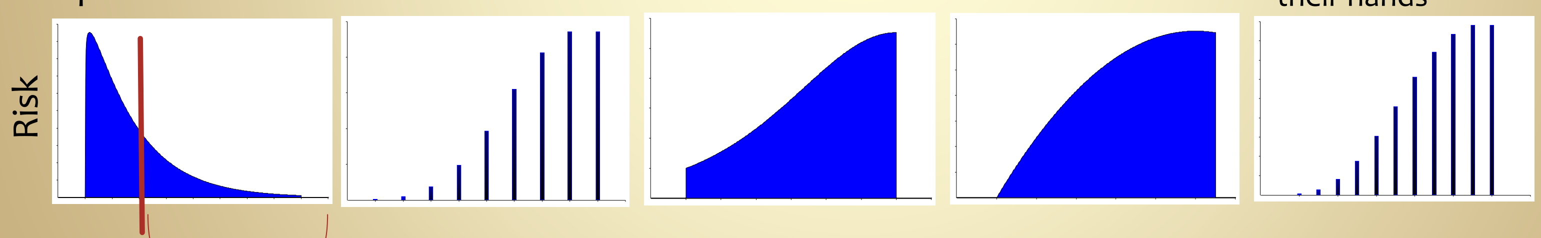
Distance to Cmm infected plants

Number of occurrences where pruning tools have Cmm

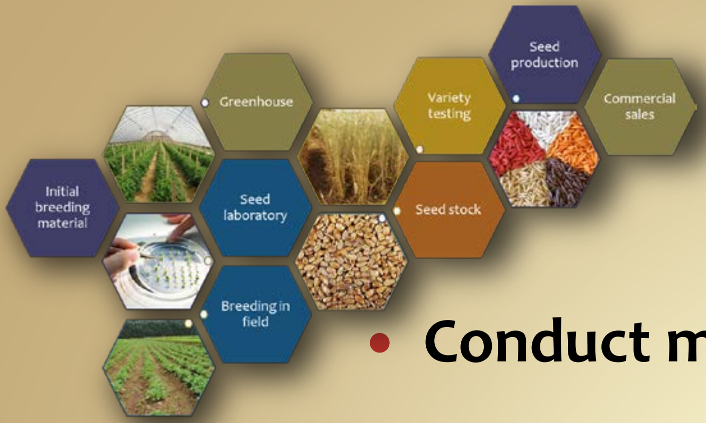
Cmm concentration in irrigation water

Plant debris (units) in nearby fields

Number of employees that forget to wash their hands

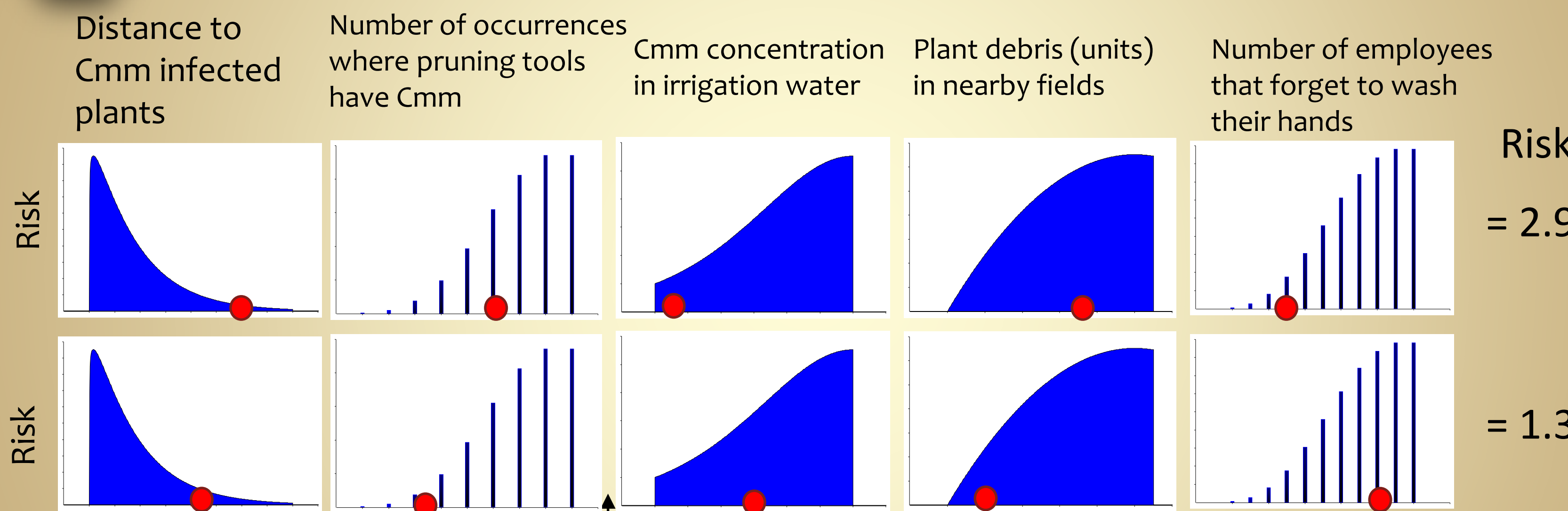



Function used to choose input data will only allow values greater than the specified distance guaranteed by Company A.

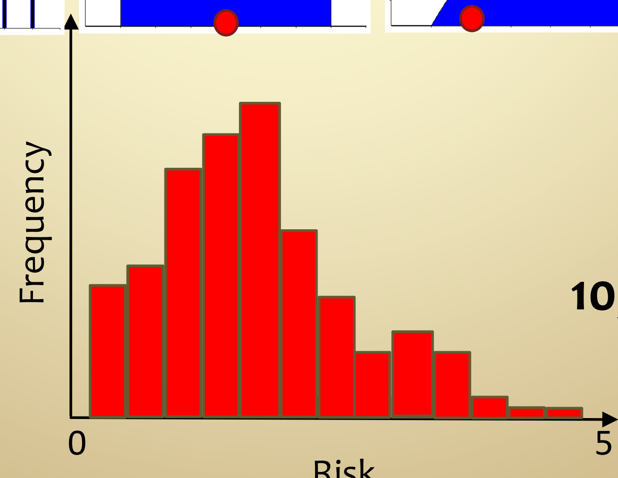



# Step 5

- **Conduct multiple iterations and examine results.**




Note: Only higher distances chosen



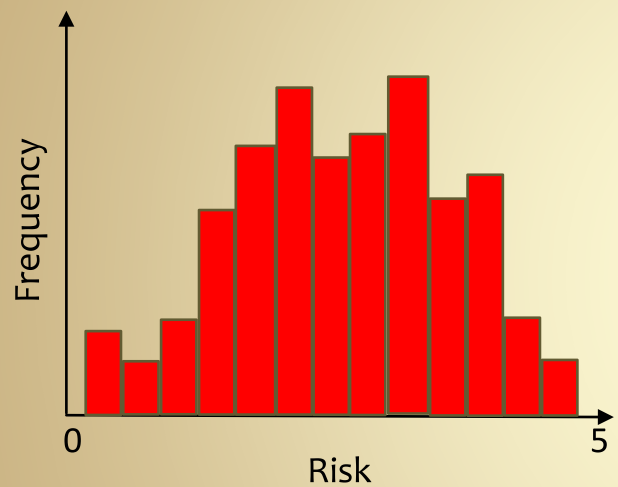

.
.
.

**10,000 iterations** = **1.8**

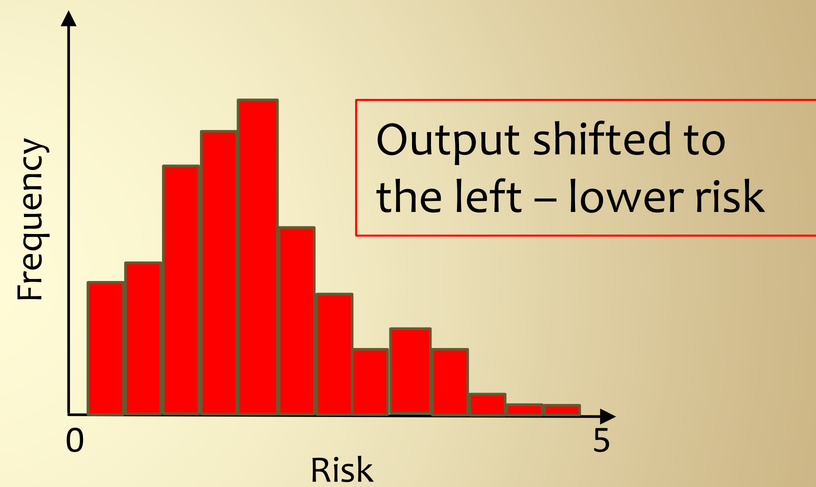
Range (0,5)

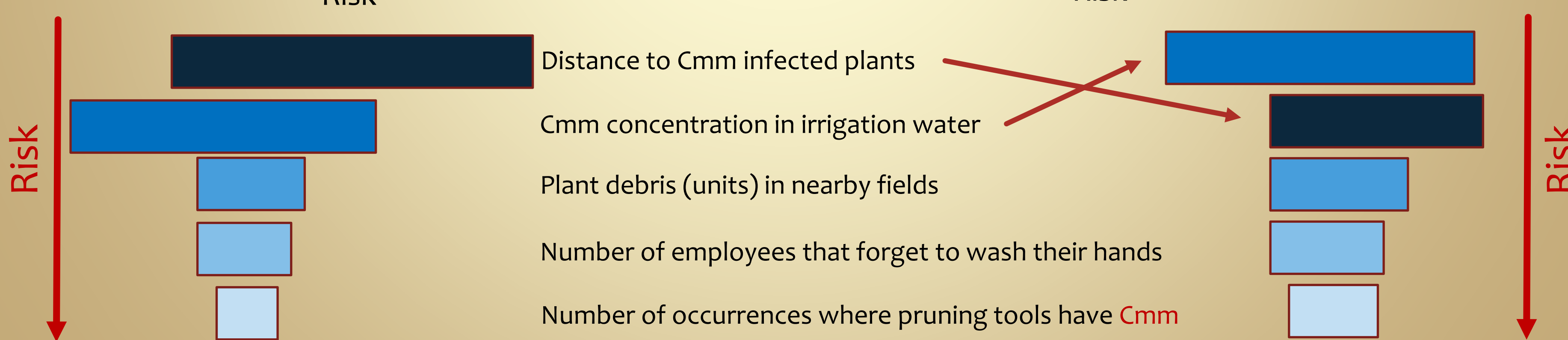
# Model application to reduce risk: 'what if' scenarios

## RISK IN A **TYPICAL** DISEASE PRESSURE ENVIRONMENT




## RISK IN A **LOW** DISEASE PRESSURE ENVIRONMENT




# Outcomes

1. Method to conduct "pathway analysis" with any plant system.
2. Method to identify phytosanitary concerns prior to large-scale (high-cost) problems.
3. Method to clearly indicate how your production practices reduce phytosanitary risks.
    1. *Assure regulatory agencies and customers 'How you stack up'!*
4. Objective "outside" scientific risk assessment. Then members of ASTA can choose to apply the assessment to their own risk management procedures.
5. Full transparency of the risk assessment communicated to ASTA.


# Project Phases: TASC Grant Funding

- **Phase 1: Aug 2014 - July 2015**
  - Acquire data on various steps in the seed production pathways for "pathosystems" Cmm and PSTVd
  - Develop risk model framework
  - Construct initial risk model and test
- **Phase 2: Aug 2015 – July 2016**
  - Model validation
  - Develop generic model if feasible
  - Deploy to ASTA and interested parties
  - Begin adapting to other pathosystems as needed/required
- **Phase 3: Aug 2016 - July 2017**
  - Deploy new model adaptations
  - Continue to develop for alternative pathosystems as requested


# What we need from you (Seed production companies)

- **Data for input distributions (*understand and appreciate that some data may be proprietary*).**
- **Goal post = Threshold levels**
  - **Set thresholds of tolerable risk at the conclusion of each pathway AND at multiple points along the pathway.**
  - **Helpful if thresholds are specified early in model development.**
  - **Mean and variability.**

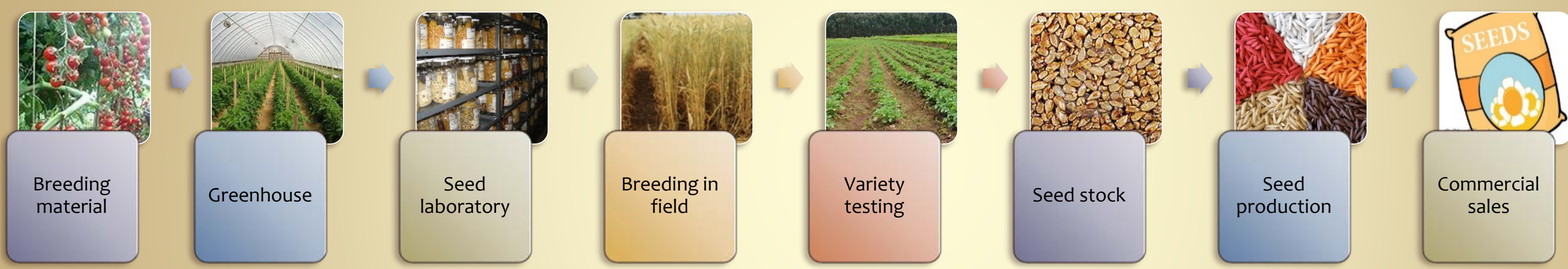
# Information needed from seed production companies!

- First we would like set up some phone discussions with key industry representatives to better understand the breadth of the systems used.
- Then we will prepare a questionnaire based on your input on specific production system information and circulate it to capture the data.

Module 3 – Laboratory or storage facility

1) Starting material
    a. Disease incidence of lot/test detection limit
    b. Effectiveness of cleaning method of receiving containers
2) Planting
    a. Is Cmm inoculation testing conducted at the location?
    b. Sanitary level where planting/handling occurs?
    c. # of times/employees forget to wash hands/equipment
    d. Water source concentration of Cmm
    e. Pathogen-free media used?
    f. Plants inspected for Cmm?
    g. Growing media and ground covers changed since last crop?
    h. Climate controlled?
        i. Temperature
        ii. Relative humidity
        iii. # hours leaf wetness per day (and after sunset)
    i. Irrigation method (overhead, drip…) coupled with volume of water during each watering
    j. Level of weed control
    k. Amount of plant debris in area
    l. During roguing, number/level of adjacent asymptomatic plants also removed
    m. Method of culling/plant disposal
        i. Piled without burying
            1. Distance of cull pile to greenhouse/production site?
        ii. Burying/composting plants
            1. Distance of cull pile to greenhouse/production site?
        iii. Others? (incineration?)
    n. Seed treatment?
        i. Hot water/dry heat
        ii. Acetic acid
        iii. Other
    o. Shipment of starting material
        i. Transport vehicles inspected/cleaned to be sanitary?
        ii. Type of transport vehicle (open, closed, controlled environment…)
        iii. Transport vehicles climate controlled (free of instances of compromised climate control integrity?) and free of moisture pockets?
        iv. Distance shipped coupled with impenetrability of shipping container to outside environment (resistance to being contaminated with Cmm)
        v. Distance of ground transportation through an area known to have Cmm hosts/infection?
        vi. Effectiveness of cleaning regime upon receipt

# Risk assessment of seed production: *From breeding to sale*




**= What Risk?**

## *Thank You for you time and attention!*

**Tim R. Gottwald, Ph.D.**
Research Leader/Plant Pathology
U.S. Dept. of Agriculture, Agricultural Research Service
Tim.Gottwald@ARS.USDA.GOV


# Budget for Probabilistic Risk assessment of seed production systems

| ARS Costs | Phase 1 2014 | Phase 2 2015 | Phase 3 2014 | 3-yr. Total |
|---|---|---|---|---|
| 1 Post Doc (model construction, validation, adaption to generic pathosystems) | $ 92,892.15 | $ 97,536.76 | $ 98,700.00 | $289,128.91 |
| GS-7 technicians (data harvesting and input) | $ 52,366.50 | $ 54,984.83 | $ 57,734.07 | $165,085.39 |
| Materials and supplies (core extension to multicore simulation computer) | $ 9,000.00 | $ 2,000.00 | $ 2,000.00 | $ 13,000.00 |
| Travel (To become familiar with various/key seed production systems and aquire data; and attend meetings to report on progress) | $ 10,000.00 | $ 5,000.00 | $ 5,000.00 | $ 20,000.00 |
| **Subtotal** | **$164,258.65** | **$159,521.58** | **$163,434.07** | **$487,214.30** |
| ARS overhead (10%) | $ 16,425.87 | $ 15,952.16 | $ 16,343.41 | $ 48,721.43 |
| **ARS Total** | **$180,684.52** | **$175,473.74** | **$179,777.47** | **$535,935.73** |

- Will likely require research on the part of other parties to fill in gaps in seed production pathway data not currently available.

# Risk assessment of seed production: breeding to sale

Greenhouse

Variety testing

Seed production

Initial breeding material

Seed laboratory

Seed stock

Commercial sales

Breeding in field

Tim R. Gottwald, Ph.D.
Research Leader/Plant Pathology
U.S. Dept. of Agriculture, Agricultural Research Service

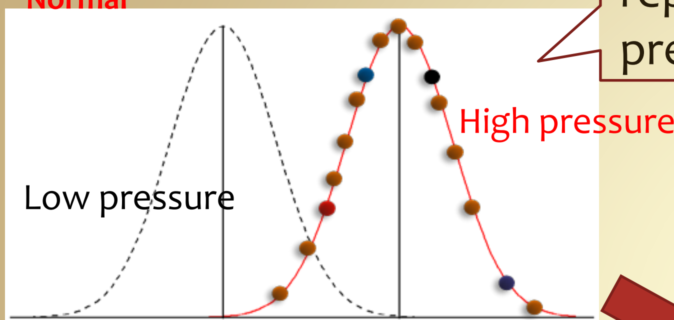
# Monte Carlo simulation

## Input variables

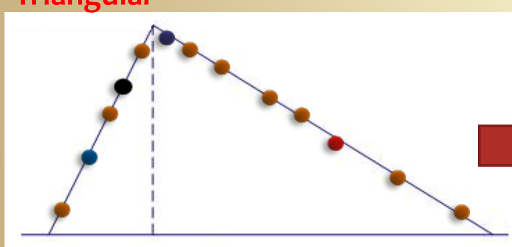
Normal distribution may represent the 'disease pressure'

## Output distribution

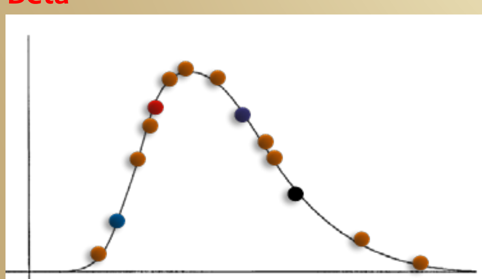
**Normal**



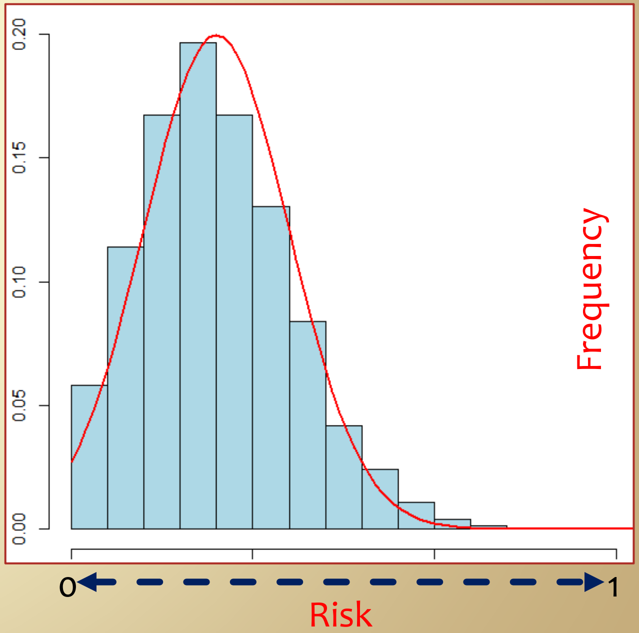

**Triangular**



**Beta**



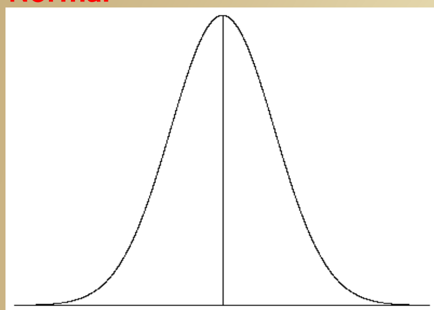
**Propagate risk from each input**



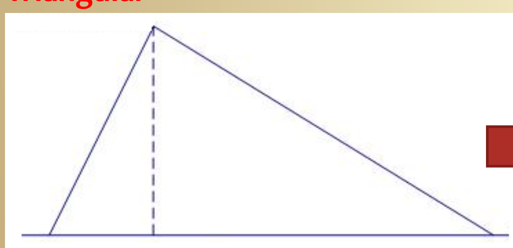
# Monte Carlo simulation

Input variables

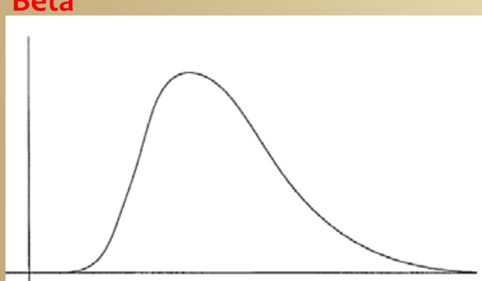
**Normal**



**Triangular**



**Beta**



**Propagate risk from each input**

Output distribution